SOME ORIENTING EFFECTS OF MONOCHROMATIC LIGHTS OF EQUAL INTENSITIES ON FUCUS SPORES AND RHIZOIDS

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One of the most striking of the biological phenomena resulting from the action of light on organisms is the orientation of the first cleavage plane of germinating spores by unilateral illumination. Whenever such illumination is sufficiently intense the first cross wall forms perpendicular to the direction of the incident light. This phenomenon has been demonstrated in Equisetum, Puccinia, Fucus and some other algae[4,8,9,10,11] together with the fact that the cell on the shaded side of the spore becomes the rhizoidal cell irrespective of gravity. Thus in these and related forms the polarity of the plant is established by the direction of light stimuli.

The power of light waves to so orient the plant is, without doubt, the power to orient the spindle of the first dividing nucleus. The mechanics of such reactions may long remain unknown; but we have a suggestive and possibly the ultimate explanation in Child's[2] metabolic gradient theory. He has demonstrated in many marine plants and in some of the lower animals the existence of the so-called 'axial gradients.' By an axial gradient is meant the decreasing rate of metabolic processes from the apical to the basal end. We may suppose that such a gradient is produced within a germinating spore whenever there is sufficient difference in the amount of light energy received on two opposite sides to produce the requisite difference in the rate of the oxidation processes along the line of direction of illumination. If Child's supposition is correct, the cell on the shaded side of the spore becomes the rhizoidal cell by virtue of the fact that the least rapid rate of the oxidation reactions along the gradient determines the basal end, the most rapid the apical end, of the organism.

The purpose of the present investigation was to study the power of pure monochromatic lights to establish the polarity of the germinating spores of *Fucus inflatus*, and also to answer several questions concerning the negative phototropism of the young rhizoids; viz., the determination of the exact wave lengths responsible for the phenomenon; the relative importance of the quality and quantity factors in the illumination or the role of intensity of illumination apart from the kind of light; and whether all effective monochromatic lights produce the same result as white light.

To obtain the monochromatic light, seven Wratten filter screens were used, each transmitting a narrow range of wave lengths but altogether embracing the whole of the visible spectrum. The wave lengths to which each screen was transparent were determined by testing the light transmitted by each with

a direct-vision spectroscope with a wave length scale attached. These screens were then fitted as windows in the ends of boxes painted black on the inside. Culture dishes were made by cementing together microscope slides, so that the light entering the boxes through the screens fell on a flat side of the dish and thus entered the water normally with a minimum loss from reflection and refraction.

The electric arc was used as the source of light wherever possible because it gives all the desired wave lengths, with the result that the whole set of screens could be used in the same exposure, insuring for all the boxes identical conditions of temperature and duration of illumination.

The spectroscopic analysis of the light transmitted by the screens gave the exact wave lengths which would act on the cultures behind them. The next step was to devise a means of making the intensities of the lights acting in each box equal so as to eliminate that most important, and hitherto largely overlooked, factor in light reactions. This done, variations in results obtained behind different screens might safely be attributed to differences in the quality of the stimulus. There have been several methods devised by which the relative intensities of monochromatic lights can be measured and made equal.[1,3,5,6,7] But all of these involve special apparatus not available for use in this investigation; so a simpler method was devised whereby the relative intensities of the lights transmitted by the Wratten filter-screens were measured by means of a thermopile and galvanometer and made equal by varying the distances of the dark boxes from the electric arc such that at these distances the deflections of the galvanometer, when the thermopile was exposed in turn to the light behind each filter screen, were equal. This distance was also measured with a piece of white glass as a filter screen which represented the removal of the control culture from the arc. The instruments used in this energy calibration were a Hilger thermopile and a d'Arsonval galvanometer.

It seems necessary on account of the questions which have been raised during the course of this work, to state here that the thermopile is equally sensitive to the energy of the red and of the violet ends of the spectrum, and is, therefore, an accurate measure of the total amount of light acting behind each color screen. The difference between heat and light is only a matter of wave length. The thermopile measures light in terms of the electric current produced by the difference in temperature of the exposed and unexposed junctions; but it does so by virtue of the fact that the energy of whatever vibrations fall upon it, be they long and therefore heating in their physiological effect, or short and therefore perceived as light, is converted into heat energy upon being absorbed by the exposed junction of the thermopile. In other words, the light of the blue end of the spectrum produces an electromotive force much less than that of the infra-red but no less measurable.

Once these distances from the arc, at which the intensities of the light in each box are equal, are determined, the quantity of light energy can be varied

by multiplying or dividing all the distances by the same multiple and the intensities in all will still remain equal to each other. By means of a photometer the actual amount of light acting in each box can then be determined by measuring the intensity in candle meters behind the white glass control at the proper distance from the arc. Then from the law of inverse squares, viz., that the intensity of light per unit surface varies inversely as the square of the distance from the source, the absolute intensity at any distance from the arc can be computed. So a Sharpe-Millar photometer was used to measure the intensity of the naked arc at the distance of the white light control. But it was then necessary to correct the measurements so obtained for the absorption of light by the glass of the filters. This so-called absorption coefficient was obtained by measuring with a Lummer Brodhum photometer the intensity of a light both with and without a screen of clear glass equal in thickness to that of the filters. It was found that glass 1.5 mm. thick absorbed 12% of the light falling upon it. Therefore to obtain the intensity of the light actually entering each dark box, it was necessary to take 88% of the reading given by the photometric measurement of the unscreened arc at the previously determined distance of the white light control.

The following table lists the colored screens used with the wave lengths they transmitted and the distances from the arc at which they were placed to make the intensity of light behind each equal to 1800 meter candles. The lack of agreement between these values and the energy curve of the spectrum is due to the individual absorption of the filters and also to the fact that they do not all transmit the same number of wave lengths.

Table showing distances at which the intensities of light from an electric arc transmitted by Wratten light filters are equal

FILTER NUMBER	WAVE-LENGTHS IN ANGSTROMS	COLOR	DISTANCE FROM LIGHT	INTENSITY IN METER CANDLES
			cm.	
70	6600–7000	Red	320	1800
71	6200–6800	Red	275	1800
72	5900–6200	Orange	230	1800
73	5600–5900	Yellow	250	1800
74	5200–5600	Green	280	1800
75	4700–5200	Blue	250	1800
76	4000–4700	Violet	250	1800
Control	4000–7000	White	340	1800

To obtain the spores of *Fucus inflatus* for the experiments, the fruiting plants were collected at low tide, kept over night in damp newspapers, and the next morning were dried slightly by exposing them to the air for about half an hour. Then when the fruiting tips were submerged in sea water in the culture dishes, large numbers of eggs and sperms were extruded and settled to the bottom of the dish. After removing the piece of plant the culture dish was placed in one of the little racks made to fit in the dark boxes

behind the filter screens. The illumination of the cultures was continued six to eight hours, this time having been found more than enough to cause the first cleavage plane to be permanently oriented regardless of subsequent illumination.

In the experiments for which the naked arc was the source of light, the heating effect was so great that the spores were killed very quickly. The mercury vapor lamp was next used to obtain wave lengths of the blue end of the spectrum and a 1000 watt nitrogen filled Tungsten globe for the red. But as in the case of the electric arc, the Tungsten light killed the spores by the high temperature produced at the distances where it was necessary to place the cultures for a sufficiently intense illumination. With the mercury vapor lamp, however, positive results were secured. The wave lengths which were found to produce the orientation of the first cleavage plane such that all the first cross walls formed perpendicular to the direction of the incident rays, are those between 4000 and 5200 Ängstrom units. Behind the two other filter screens used with the mercury vapor lamp and transmitting wave lengths of 5200 to 5900 Ängstrom units, the spores germinated as if in darkness with the orientations of the first cleavage planes following no rule, and the rhizoids extending in all directions. However, the intensities of the lights behind these color screens were not equal when the mercury lamp was used because the shorter blue wave lengths predominated to so great a degree and hence produced greater intensities.

With regard to the phototropism of the young rhizoids, it was found that very weak white light, too weak to orient the cleavage planes, would cause the growing tips to turn sharply away from the source of light. With the intensity of illumination behind all the color screens equal to 1800 meter candles, only the blue and violet lights produced the phototropism. The other wave lengths at this intensity had no effect, the young rhizoids continuing in the direction in which they had started just as did those of the control in darkness. However when a more intense illumination was secured by placing the boxes in direct sunlight, the rhizoids behind the green filter, in addition to those behind the blue and violet ones, showed the same negative phototropism. This and subsequent experiments would lead us to believe that both quantity and quality, or intensity and wave length, are determining factors in the power of light stimuli to produce phototropisms.

In every culture of *Fucus inflatus* whether germinated in darkness or in strong unilateral light a most striking orientation of the first cross-wall with reference to adjacent spores appears. Wherever a group of spores are lying within about 0.2 mm. of each other, the first cleavage plane is perpendicular to the direction of the center of the group. The cell toward the interior invariably becomes the rhizoidal cell. This phenomenon was reported by Rosenvinge[9] in other species of *Fucus* and in *Ascophyllum*. For want of a better term I have called it *group orientation*. A study of the phenomenon was made to determine the strength of this stimulus, compared to that of

light, in its power to establish the orientation of the plant. It was at once very evident that for most spores the former stimulation is stronger when the spores are within a short distance of each other—0.2 mm. or often more—but beyond this distance, the chemical stimulus becomes too weak and only the light is able to determine the polarity of the plant. Only the comparatively isolated spores therefore show the orientation to light with the sources of illumination used here.

The phenomenon is very conspicuous in groups of 2, 3, or 4 eggs as well as in masses of 50 or 100. In these large groups it is made evident by the invariable rule that no rhizoid ever extends outward from a group. When two spores are within the distance through which the stimulus is effective, the first cleavage planes of the two are parallel and the rhizoids grow towards each other and often meet tip to tip. The groups of 5 or 6 often make symmetrical star-like designs when the rhizoids have grown and project beyond the group. The spores are more rarely affected in this way when the distance between them is over 0.3 mm. but the phenomenon is sometimes observed in spores as much as 0.5 mm. apart. Within a distance of 0.2 mm. there are practically no exceptions.

The relative sensitiveness of a spore towards light and towards this chemical (?) stimulus varies greatly for different spores. When cultures were placed in the window to get as strong a light stimulus as possible in order to determine at what distance from each other the eggs had to be not to show a greater sensitiveness towards the chemical stimulus than towards the light, it was found that this distance followed no rule, the spores showing the greatest individual differences. Of two spores lying within 0.3 mm. of each other one might be entirely oriented by the adjacent spore while the other, apparently like it, would show only the action of the light stimulus. In many cases two such spores would seem to show a resultant effect of the two stimuli so that both would be half turned towards each other with both rhizoidal cells showing a tendency to take a direction away from the light at the same resultant angle.

Rosenvinge ascribes this group orientation to a difference in the concentration of oxygen or of nutritive substances on the two sides of the spore. He thinks the rhizoid forms on the side toward the center of a group or towards another egg because the water on that side is less rich in the active substance than on the outer side as a result of their metabolism. Winkler[11] working with *Cystoseira barbata* found that a difference in oxygen concentration has no such effect. Apparently the phenomenon does not occur naturally in this species since his figure shows nothing but the effect of light. I have never seen a culture of *Fucus inflatus* with spores germinating so near each other, which showed only light orientation and not the group orientation. Almost invariably when the spores of this species germinate in such close proximity, light appears to have no power to establish the polarity of the plant.

The possibility that the group orientation is due to a polarity established by the position of the egg in the oogonium is suggested by finding many

groups of eight lying just as they escaped from the oogonial sac and conspicuously oriented with respect to each other. The fact that groups of ten or of two are as regularly oriented, would refute the suggestion; but in order to prove that the phenomenon is the result of a stimulus acting after the eggs leave the oogonium, a group of them were transferred to a watch crystal and mixed with the point of a needle until their relative positions were entirely changed. But when they germinated the characteristic orientation with respect to each other was found to be without an exception.

The only apparent explanation of the group orientation is that of a diffusion gradient of some substance emanating from a growing spore, or of some substance being used up by it. A continuation of the investigation of this problem will be an attempt to discover a substance which can so affect the dividing nucleus of the egg cell that its unequal distribution on the sides of the cell will orient the axis of the spindle. The effect of bubbling carbon dioxide and oxygen through cultures should be tried as being the most probable factors involved.

The substance or condition originating in the activity of adjacent spores which has so powerful an effect in orienting the first cleavage plane and in determining which cell shall become the rhizoidal cell has no power to cause any chemotropism of the rhizoids after they are started. No rhizoid has been found to have its direction modified by the presence of other spores adjacent to it. In the absence of any light stimulus the rhizoids continue in the direction that they take originally from the spore.

[1] Blaaw, A. H., *Rec. Travaux Bot. Néerlandais*, **5**, 1908, (209–372).
[2] Child, C. M., *Individuality of Organisms*, 1915.
[3] Day, E. C., *Bull. Mus. Comp. Zool.* **53**, 1911, (303–343).
[4] Farmer, J. B., and Williams, J. L., *Proc. Roy. Soc.*, **60**, 1896, (188–195).
[5] Kniep, G. and Minder, F., *Zs. Bot.*, **1**, 1909, (619).
[6] MacDougal, D. T. and Spoehr, H. A., *Science*, (N. S.), **45**, 1917, (616–618).
[7] Mast, S. O., *J. Comp. Neur. Psych.*, **17**, 1907, (99–179).
[8] Randolph, F. A., and Peirce, G. J., *Bot. Gaz.*, **40**, 1905, (321–350).
[9] Rosenvinge, M. L. K., *Revue Gen. Bot.*, **1**, 1889, (125–135).
[10] Stahl, E., *Ber. deuts. bot. Ges.*, **3**, 1885, (334–340).
[11] Winkler, H., *Ibid.*, **18**, 1900, (297–305).

ON THE MOST GENERAL CLASS L OF FRÉCHET IN WHICH THE HEINE-BOREL-LEBESGUE THEOREM HOLDS TRUE

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§1 A class L of Fréchet[1] is a set of elements such that (1) if P is an element of L and $P_1, P_2, P_3 \ldots$ is a countable[2] sequence of elements belonging to L then the statement that P is the limit of the sequence $P_1, P_2, P_3, \ldots$